



Data Paper

# The InBIO barcoding initiative database: DNA barcodes of Iberian Trichoptera, documenting biodiversity for freshwater biomonitoring in a Mediterranean hotspot

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## Abstract

### Background

The Trichoptera are an important component of freshwater ecosystems. In the Iberian Peninsula, 380 taxa of caddisflies are known, with nearly 1/3 of the total species being endemic in the region. A reference collection of morphologically identified Trichoptera specimens, representing 142 Iberian taxa, was constructed. The InBIO Barcoding Initiative (IBI) Trichoptera 01 dataset contains records of 438 sequenced specimens. The species of this dataset correspond to about 37% of Iberian Trichoptera species diversity. Specimens were collected between 1975 and 2018 and are deposited in the IBI collection at the CIBIO (Research Center in Biodiversity and Genetic Resources, Portugal) or in the collection Marcos A. González at the University of Santiago de Compostela (Spain).

### New information

Twenty-nine species, from nine different families, were new additions to the Barcode of Life Data System (BOLD). A success identification rate of over 80% was achieved when comparing morphological identifications and DNA barcodes for the species analysed. This encouraging step advances incorporation of informed Environmental DNA tools in biomonitoring schemes, given the shortcomings of morphological identifications of larvae and adult Caddisflies in such studies. DNA barcoding was not successful in identifying species in six Trichoptera genera: *Hydropsyche* (Hydropsychidae), *Athripsodes* (Leptoceridae), *Wormaldia* (Philopotamidae), *Polycentropus* (Polycentropodidae) *Rhyacophila* (Rhyacophilidae) and *Sericostoma* (Sericostomatidae). The high levels of intraspecific genetic variability found, combined with a lack of a barcode gap and a challenging morphological identification, rendered these species as needing additional studies to resolve their taxonomy.

### Keywords

Trichoptera, occurrence records, species distributions, continental Portugal, continental Spain, DNA barcode, cytochrome c oxidase subunit I (COI)

### Introduction

DNA barcoding is a molecular biology method for species identification that was proposed almost twenty years ago (Hebert et al. 2003). DNA barcoding relies on the comparison of a short mitochondrial DNA sequence of interest, usually a 658 bp fragment of the cytochrome c oxidase subunit I (COI) of the mitochondrial genome, known as the “Folmer region” (Folmer et al. 1994), although other regions and genes can also be used, including ones with different systematic scopes (e.g. Woese and Fox (1977)). For DNA barcoding to



work, the sequence of interest must be compared to a library containing sequences with known species identification (Hebert et al. 2003, Hebert et al. 2004). As such, the construction of comprehensive reference libraries is essential and these require the morphological identification of vouchers by an expert taxonomist (Baird and Sweeney 2011, Ferreira et al. 2018, Kress et al. 2015). DNA barcoding applications have since expanded beyond single organism and species identification studies.

Development of DNA metabarcoding (Taberlet et al. 2012) was made possible with the advances in PCR technologies and high-throughput sequencing (HTS) (Liu et al. 2019). Multiple DNA barcodes are sequenced in a single sample, allowing the study of complex samples as bulk samples and environmental DNA. DNA metabarcoding has broadened the use of the two. DNA barcodes are now a ubiquitous tool in ecological and biological conservation studies, as well as, for example, in forensic applications (DeSalle and Goldstein 2019, Fišer Pečnikar and Buzan 2013, Kress et al. 2015).

Aquatic ecosystems are suffering high losses in biodiversity due to degradation and habitat destruction (Blancher et al. 2022). These ecosystems can be logistically challenging and time-consuming to monitor, as the current methodology is based on inventories and taxonomical diversity, based on morphology (Blancher et al. 2022). DNA metabarcoding has great potential for conservation and monitoring of aquatic ecosystems studies as it allows efficient, non-invasive and standardised sampling, without a priori knowledge of the existing biodiversity in an area (Thomsen and Willerslev 2015, Valentini et al. 2016). The choice of DNA markers and the biomass of the communities to monitor are important factors that can influence successful use of DNA metabarcoding (Thomsen and Willerslev 2015, Valentini et al. 2016, Casey et al. 2021).

The Trichoptera, or caddisflies, is an order of holometabolous insects that rank seventh overall amongst insect orders regarding species number, with 16,267 described species (Morse 2022) and is the most speciose of the primarily aquatic insect orders. Species of this order can be found in all continents, except Antarctica (Morse et al. 2019). While adults are mostly terrestrial and capable of flight, most species' eggs, larvae and pupae are found in freshwater habitats (Morse et al. 2019). Adult caddisflies are moth-like insects having their bodies covered with setae or hairs (Holzenthal et al. 2007, Morse et al. 2019, Thomas et al. 2020). Their larvae are known for their ability to use silk to construct shelters and retreats, but some species can also be free-living (Casey et al. 2021, González and Cobo 2006, Holzenthal et al. 2015, Martín 2017, Martínez 2014, Morse et al. 2019, Thomas et al. 2020, Zhou et al. 2016). Caddisfly larvae provide several important ecological services, including their crucial role in the trophic dynamics and energy flow in the lakes, rivers and streams freshwater food webs (Holzenthal et al. 2015, Morse et al. 2019, Zhou et al. 2016). They show differential sensitivity to pollution and their diversity and abundance are widely used in biological freshwater monitoring (Resh and Rosenberg 1984). However, these programmes rely on larval morphological identification, which is much more challenging than adult determination and still impossible in the many species, whose larvae have not yet been described (Morse et al. 2019).



Environmental DNA has the potential to be used as a complement or as an alternative to the hurdles of current morphology-based identification in the scope of freshwater monitoring schemes (Lefrançois et al. 2020). However, successful application of eDNA in Europe will necessitate comprehensive reference collections of DNA sequences, representing existing European aquatic biodiversity (Baird and Sweeney 2011, Ferreira et al. 2018, Kress et al. 2015). Several studies have used barcodes to advance the knowledge on Trichoptera, either expanding the knowledge on their phylogeny or improving the DNA barcodes of Trichoptera species (e.g. Morinière et al. (2017), Zhou et al. (2016)).

In the Iberian Peninsula, approximately 380 Trichoptera taxa, from 23 families are known (Coppa et al. 2022, González et al. 1992, González and Martínez 2011, Malicky 2005, Martín 2017, Martínez 2014, Oláh et al. 2019a, Oláh et al. 2019b, Oláh et al. 2020, Valladolid et al. 2018, Titos et al. 2018). Of these, 374 are known in Spain and 190 in Portugal. The rate of endemism of Iberian caddisflies is very high, with around one third of the taxa known to occur in the region being endemic (González et al. 1987, Martínez 2014, Martín 2017).

In this work, we present a contribution to the DNA barcode library of the Iberian Peninsula species of Trichoptera representing 37% ( $n = 142$ ) of the Caddisflies known in the region and 38% ( $n = 57$ ) of the known endemic Iberian taxa. This work was conducted within the framework of the InBIO Barcoding Initiative.

## General description

**Purpose:** This dataset aims to provide a first contribution to an authoritative DNA barcode sequences library for Iberian Trichoptera, documenting biodiversity for freshwater biomonitoring in a Mediterranean hotspot. Such a library aims to enable DNA-based identification of species for both traditional molecular studies and DNA-metabarcoding studies. Furthermore, it constitutes a relevant resource for taxonomic research on Iberian Trichoptera and its distribution.

**Additional information:** A total of 438 Trichoptera specimens were sequenced (Suppl. material 1). A full-length barcode of 658 bp was obtained for 400 specimens (91.3%) (Table 1, Suppl. material 2). These specimens represent 142 (37%) of the approximately 380 Caddisflies species known to occur in the Iberian Peninsula (González and Martínez 2011, Martínez 2014, Martín 2017). Furthermore, 57 taxa are Iberian endemics, representing 38% of the total endemic Iberian taxa (González and Martínez 2011, Martínez 2014, Martín 2017). The dataset includes 22 of the 23 families known to occur in the Iberian Peninsula (Table 1). These data contribute with 29 new taxa, 26 new species and three new subspecies of Trichoptera to the BOLD database (Table 1). For five additional species, the dataset contributes for the first time a full-length barcode.



Table 1.

List of species that were collected and DNA barcoded within this project.

Family	Taxa	IBI code	BOLD code	BOLD BIN
Apataniidae	<i>Apatania theischingerorum</i> Malicky, 1981	INV05962	<a href="#">IBITR421-20</a>	<a href="#">BOLD:ADL7734</a>
Beraeidae	<i>Beraea alva</i> Malicky, 1975	INV05488	<a href="#">IBITR348-20</a>	<a href="#">BOLD:AAJ8091</a>
	<i>Beraea malatebrera</i> Schmid, 1952	INV04753	<a href="#">IBITR267-20</a>	<a href="#">BOLD:AAO2491</a>
		INV04267	<a href="#">IBITR173-20</a>	
Brachycentridae	<i>Micrasema cenerentola</i> Schmid, 1952	INV05952	<a href="#">IBITR414-20</a>	<a href="#">BOLD:AAO3157</a>
	<i>Micrasema longulum</i> McLachlan, 1876	INV06484	<a href="#">IBITR435-20</a>	<a href="#">BOLD:AAK7456</a>
	<i>Micrasema minimum</i> McLachlan, 1876	INV05973	<a href="#">IBITR430-20</a>	<a href="#">BOLD:AAH6898</a>
		INV05974	<a href="#">IBITR431-20</a>	
	<i>Micrasema moestum</i> (Hagen, 1868)	INV00475	<a href="#">IBITR054-20</a>	<a href="#">BOLD:AAO1660</a>
		INV00476	<a href="#">IBITR055-20</a>	
		INV00477	<a href="#">IBITR056-20</a>	
	<i>Micrasema servatum</i> (Navás, 1918)	INV04731	<a href="#">IBITR252-20</a>	<a href="#">BOLD:AAH3018</a>
		INV04732	<a href="#">IBITR253-20</a>	
Calamoceratidae	<i>Calamoceras marsupus</i> Brauer, 1865	INV02470	<a href="#">IBITR101-20</a>	<a href="#">BOLD:AAO2482</a>
		INV05476	<a href="#">IBITR336-20</a>	
Ecnomidae	<i>Ecnomus deceptor</i> McLachlan, 1884	INV03539	<a href="#">IBITR140-20</a>	<a href="#">BOLD:ABU6618</a>
		INV03546	<a href="#">IBITR141-20</a>	
		INV03605	<a href="#">IBITR146-20</a>	
		INV05502	<a href="#">IBITR360-20</a>	
Glossosomatidae	<i>Agapetus delicatulus</i> McLachlan, 1884	INV05812	<a href="#">IBITR388-20</a>	<a href="#">BOLD:AAE6313</a>
		INV05813	<a href="#">IBITR389-20</a>	
		INV05814	<a href="#">IBITR390-20</a>	<a href="#">BOLD:AAE6313</a>
	<i>Agapetus fuscipes</i> Curtis, 1834	INV05815	<a href="#">IBITR391-20</a>	<a href="#">BOLD:AAJ7120</a>
		INV05816	<a href="#">IBITR392-20</a>	
		INV02468	<a href="#">IBITR099-20</a>	<a href="#">BOLD:AEC9758</a>
		INV05817	<a href="#">IBITR393-20</a>	<a href="#">BOLD:AAJ7120</a>
	<i>Agapetus incertulus</i> McLachlan, 1884	INV04759	<a href="#">IBITR273-20</a>	<a href="#">BOLD:AEC9946</a>
	<i>Agapetus nimbulus</i> McLachlan, 1879	INV04762	<a href="#">IBITR274-20</a>	<a href="#">BOLD:AEM2297</a>

Family	Taxa	IBI code	BOLD code	BOLD BIN
	<i>Agapetus ochripes</i> Curtis, 1834	INV04763	<a href="#">IBITR275-20</a>	<a href="#">BOLD:AAB3823</a>
	<i>Agapetus segovicus</i> Schmid, 1952	INV05818	<a href="#">IBITR394-20</a>	<a href="#">BOLD:AEC7102</a>
		INV05819	<a href="#">IBITR395-20</a>	
		INV01163	<a href="#">IBITR075-20</a>	<a href="#">BOLD:AEC7102</a>
		INV04819	<a href="#">IBITR317-20</a>	
	<i>Agapetus theischingeri</i> Malicky, 1980	INV05823	<a href="#">IBITR396-20</a>	<a href="#">BOLD:AEL9298</a>
	<i>Catagapetus macLachlani</i> Malicky, 1975	INV05826	<a href="#">IBITR399-20</a>	<a href="#">BOLD:ABA7173</a>
		INV02477	<a href="#">IBITR108-20</a>	
		INV02929	<a href="#">IBITR127-20</a>	
		INV03936	<a href="#">IBITR169-20</a>	
		INV05824	<a href="#">IBITR397-20</a>	
		INV05825	<a href="#">IBITR398-20</a>	
	<i>Glossosoma privatum</i> McLachlan, 1884	INV05831	<a href="#">IBITR401-20</a>	<a href="#">BOLD:AAM0930</a>
		INV00461	<a href="#">IBITR050-20</a>	
		INV00468	<a href="#">IBITR052-20</a>	
		INV04688	<a href="#">IBITR219-20</a>	
		INV04689	<a href="#">IBITR220-20</a>	
		INV05830	<a href="#">IBITR400-20</a>	
	<i>Synagapetus diversus</i> (McLachlan, 1884)	INV05491	<a href="#">IBITR351-20</a>	<a href="#">BOLD:ABX9025</a>
	<i>Synagapetus lusitanicus</i> Malicky, 1980	INV05833	<a href="#">IBITR402-20</a>	<a href="#">BOLD:AAO4326</a>
Goeridae	<i>Larcasia partita</i> Navás, 1917	INV00320	<a href="#">IBITR027-20</a>	<a href="#">BOLD:AEC6981</a>
		INV00327	<a href="#">IBITR029-20</a>	
		INV00329	<a href="#">IBITR030-20</a>	
		INV00341	<a href="#">IBITR034-20</a>	
		INV00451	<a href="#">IBITR047-20</a>	
		INV02473	<a href="#">IBITR104-20</a>	
		INV02474	<a href="#">IBITR105-20</a>	
		INV04733	<a href="#">IBITR254-20</a>	
	<i>Silo graellsii</i> Pictet, 1865	INV05951	<a href="#">IBITR413-20</a>	<a href="#">BOLD:AEC7954</a>
Helicopsychidae	<i>Helicopsyche lusitanica</i> McLachlan, 1884	INV00005	<a href="#">IBITR012-20</a>	<a href="#">BOLD:AEC8414</a>



Family	Taxa	IBI code	BOLD code	BOLD BIN
	<i>Helicopsyche</i> sp.	INV04823	<a href="#">IBITR321-20</a>	<a href="#">BOLD:AED0915</a>
		INV04824	<a href="#">IBITR322-20</a>	<a href="#">BOLD:AEC8747</a>
		INV04825	<a href="#">IBITR323-20</a>	
Hydropsychidae	<i>Cheumatopsyche lepida</i> (Pictet, 1834)	INV04778	<a href="#">IBITR283-20</a>	<a href="#">BOLD:AAD1893</a>
		INV04779	<a href="#">IBITR284-20</a>	
		INV06149	<a href="#">IBITR432-20</a>	
		INV06590	<a href="#">IBITR436-20</a>	
	<i>Diplectrona felix</i> McLachlan, 1878	INV04718	<a href="#">IBITR243-20</a>	<a href="#">BOLD:AAO2443</a>
		INV04719	<a href="#">IBITR244-20</a>	
		INV05479	<a href="#">IBITR339-20</a>	
		INV05957	<a href="#">IBITR418-20</a>	
	<i>Hydropsyche ambigua</i> Schmid, 1973	INV03635	<a href="#">IBITR151-20</a>	<a href="#">BOLD:AAB5092</a>
		INV05480	<a href="#">IBITR340-20</a>	<a href="#">BOLD:AAB9587</a>
		INV05960	<a href="#">IBITR420-20</a>	
		INV04720	<a href="#">IBITR245-20</a>	
		INV04721	<a href="#">IBITR246-20</a>	
		INV05493	<a href="#">IBITR353-20</a>	
	<i>Hydropsyche brevis</i> Mosely, 1930	INV04780	<a href="#">IBITR285-20</a>	<a href="#">BOLD:AEC9027</a>
		INV04781	<a href="#">IBITR286-20</a>	
	<i>Hydropsyche bulbifera</i> McLachlan, 1878	INV04783	<a href="#">IBITR288-20</a>	<a href="#">BOLD:AAO1831</a>
		INV00809	<a href="#">IBITR071-20</a>	
		INV04503	<a href="#">IBITR190-20</a>	
		INV04782	<a href="#">IBITR287-20</a>	
	<i>Hydropsyche dinarica</i> Marinkovic-Gospodnetic, 1979	INV05956	<a href="#">IBITR417-20</a>	<a href="#">BOLD:AAE5138</a>
	<i>Hydropsyche exocellata</i> Dufour, 1841	INV02678	<a href="#">IBITR111-20</a>	<a href="#">BOLD:AAF0933</a>
		INV04785	<a href="#">IBITR289-20</a>	
		INV00433	<a href="#">IBITR045-20</a>	
		INV00434	<a href="#">IBITR046-20</a>	
		INV02920	<a href="#">IBITR121-20</a>	
		INV02922	<a href="#">IBITR123-20</a>	

Family	Taxa	IBI code	BOLD code	BOLD BIN
		INV02979	<a href="#">IBITR135-20</a>	
	<i>Hydropsyche iberomaroccana</i> González & Malicky, 1999	INV04788	<a href="#">IBITR292-20</a>	<a href="#">BOLD:AED0538</a>
	<i>Hydropsyche infernalis</i> Schmid, 1952	INV04789	<a href="#">IBITR293-20</a>	<a href="#">BOLD:AAB5092</a>
	<i>Hydropsyche instabilis</i> (Curtis, 1834)	INV04722	<a href="#">IBITR247-20</a>	<a href="#">BOLD:AAB1966</a>
		INV04723	<a href="#">IBITR248-20</a>	<a href="#">BOLD:ABZ1867</a>
		INV05959	<a href="#">IBITR419-20</a>	<a href="#">BOLD:AAB1966</a>
	<i>Hydropsyche lobata</i> McLachlan, 1884	INV04501	<a href="#">IBITR189-20</a>	<a href="#">BOLD:AEC7586</a>
		INV04787	<a href="#">IBITR291-20</a>	
		INV00561	<a href="#">IBITR069-20</a>	
		INV02669	<a href="#">IBITR110-20</a>	
		INV03591	<a href="#">IBITR144-20</a>	
		INV03592	<a href="#">IBITR145-20</a>	
		INV04786	<a href="#">IBITR290-20</a>	
	<i>Hydropsyche pictetorum</i> Botosaneanu & Schmid, 1973	INV00421	<a href="#">IBITR037-20</a>	<a href="#">BOLD:AAO2260</a>
		INV02962	<a href="#">IBITR132-20</a>	<a href="#">BOLD:AAB5092</a>
		INV04790	<a href="#">IBITR294-20</a>	<a href="#">BOLD:AAO2260</a>
		INV05505	<a href="#">IBITR363-20</a>	
	<i>Hydropsyche siltalai</i> Doehler, 1963	INV04269	<a href="#">IBITR175-20</a>	<a href="#">BOLD:AAB5092</a>
		INV00186	<a href="#">IBITR025-20</a>	
		INV00460	<a href="#">IBITR049-20</a>	
		INV04724	<a href="#">IBITR249-20</a>	<a href="#">BOLD:AAB9587</a>
		INV03680	<a href="#">IBITR160-20</a>	<a href="#">BOLD:AAB5092</a>
		INV05481	<a href="#">IBITR341-20</a>	
		INV05482	<a href="#">IBITR342-20</a>	
		INV05494	<a href="#">IBITR354-20</a>	
	<i>Hydropsyche tenuis</i> Navás, 1932	INV00318	<a href="#">IBITR026-20</a>	<a href="#">BOLD:AAB9587</a>
	<i>Hydropsyche tibialis</i> McLachlan, 1884	INV04725	<a href="#">IBITR250-20</a>	<a href="#">BOLD:AED0962</a>
		INV04726	<a href="#">IBITR251-20</a>	
		INV06211	<a href="#">IBITR433-20</a>	
Hydroptilidae	<i>Agraylea sexmaculata</i> Curtis, 1834	INV04524	<a href="#">IBITR192-20</a>	<a href="#">BOLD:AAE7232</a>



Family	Taxa	IBI code	BOLD code	BOLD BIN
		INV02924	<a href="#">IBITR124-20</a>	
		INV02927	<a href="#">IBITR125-20</a>	
		INV02928	<a href="#">IBITR126-20</a>	
		INV03549	<a href="#">IBITR142-20</a>	
	<i>Hydroptila fuentaldeala</i> Schmid, 1952	INV05477	<a href="#">IBITR337-20</a>	<a href="#">BOLD:AEC8395</a>
	<i>Ithytrichia clavata</i> Morton, 1905	INV00520	<a href="#">IBITR066-20</a>	<a href="#">BOLD:AEC8346</a>
		INV00839	<a href="#">IBITR073-20</a>	
	<i>Oxyethira frici</i> Klapalek, 1891	INV05503	<a href="#">IBITR361-20</a>	<a href="#">BOLD:ABY2898</a>
Lepidostomatidae	<i>Lepidostoma hirtum</i> (Fabricius, 1775)	INV00009	<a href="#">IBITR002-16</a>	<a href="#">BOLD:AAB4052</a>
		INV00010	<a href="#">IBITR003-16</a>	
		INV00011	<a href="#">IBITR004-16</a>	
		INV04579	<a href="#">IBITR202-20</a>	
		INV04584	<a href="#">IBITR204-20</a>	
Leptoceridae	<i>Adicella meridionalis</i> Morton, 1906	INV05510	<a href="#">IBITR367-20</a>	<a href="#">BOLD:AEM0162</a>
	<i>Adicella reducta</i> (McLachlan, 1865)	INV00422	<a href="#">IBITR038-20</a>	<a href="#">BOLD:AAJ1835</a>
		INV00426	<a href="#">IBITR041-20</a>	
		INV00012	<a href="#">IBITR005-16</a>	
		INV00013	<a href="#">IBITR006-16</a>	
		INV00014	<a href="#">IBITR007-16</a>	
		INV00470	<a href="#">IBITR053-20</a>	
		INV00482	<a href="#">IBITR058-20</a>	
		INV02475	<a href="#">IBITR106-20</a>	
		INV04856	<a href="#">IBITR325-20</a>	
	<i>Athripsodes alentexanus</i> Martín, González & Martínez, 2016	INV06592	<a href="#">IBITR437-20</a>	<a href="#">BOLD:AAI7978</a>
		INV06593	<a href="#">IBITR438-20</a>	
	<i>Athripsodes braueri</i> (Pictet, 1865)	INV04268	<a href="#">IBITR174-20</a>	
		INV05485	<a href="#">IBITR345-20</a>	
	<i>Athripsodes inaequalis</i> (McLachlan, 1884)	INV02463	<a href="#">IBITR094-20</a>	<a href="#">BOLD:AED0841</a>
		INV02919	<a href="#">IBITR120-20</a>	
		INV03273	<a href="#">IBITR137-20</a>	

Family	Taxa	IBI code	BOLD code	BOLD BIN
	<i>Athripsodes tavaresi</i> (Navás, 1916)	INV02764	<a href="#">IBITR116-20</a>	<a href="#">BOLD:AEC8026</a>
		INV03612	<a href="#">IBITR147-20</a>	
		INV04754	<a href="#">IBITR268-20</a>	
	<i>Ceraclea albimacula</i> (Rambur, 1842)	INV00184	<a href="#">IBITR024-20</a>	<a href="#">BOLD:AAN2950</a>
		INV02233	<a href="#">IBITR084-20</a>	
		INV04556	<a href="#">IBITR197-20</a>	<a href="#">BOLD:AAD8966</a>
	<i>Ceraclea sobradieli</i> (Navás, 1917)	INV02950	<a href="#">IBITR001-16</a>	<a href="#">BOLD:AAD8965</a>
		INV02948	<a href="#">IBITR128-20</a>	
		INV04554	<a href="#">IBITR196-20</a>	
		INV04510	<a href="#">IBITR191-20</a>	
		INV05474	<a href="#">IBITR334-20</a>	
		INV05484	<a href="#">IBITR344-20</a>	
	<i>Leptocerus tineiformis</i> Curtis, 1834	INV00812	<a href="#">IBITR072-20</a>	<a href="#">BOLD:AAJ1160</a>
		INV00846	<a href="#">IBITR074-20</a>	
		INV04287	<a href="#">IBITR177-20</a>	
	<i>Mystacides azureus</i> (Linnaeus, 1761)	INV02239	<a href="#">IBITR085-20</a>	<a href="#">BOLD:AAB1494</a>
		INV03563	<a href="#">IBITR143-20</a>	
		INV04818	<a href="#">IBITR316-20</a>	
	<i>Oecetis testacea</i> (Curtis, 1834)	INV05473	<a href="#">IBITR333-20</a>	<a href="#">BOLD:AAD7208</a>
	<i>Setodes argentipunctellus</i> McLachlan, 1877	INV05352	<a href="#">IBITR327-20</a>	<a href="#">BOLD:ACB2223</a>
		INV05353	<a href="#">IBITR328-20</a>	
		INV00549	<a href="#">IBITR068-20</a>	
		INV04817	<a href="#">IBITR315-20</a>	
	<i>Triaenodes ochreellus</i> McLachlan, 1877	INV02467	<a href="#">IBITR098-20</a>	<a href="#">BOLD:AAJ8708</a>
Limnephilidae	<i>Allogamus laureatus</i> (Navás, 1918)	INV02246	<a href="#">IBITR086-20</a>	<a href="#">BOLD:AEC7060</a>
	<i>Allogamus ligonifer</i> (McLachlan, 1876)	INV00321	<a href="#">IBITR028-20</a>	<a href="#">BOLD:AAO2353</a>
		INV02462	<a href="#">IBITR093-20</a>	
		INV02466	<a href="#">IBITR097-20</a>	
		INV04748	<a href="#">IBITR264-20</a>	
		INV03724	<a href="#">IBITR164-20</a>	



Family	Taxa	IBI code	BOLD code	BOLD BIN
		INV03727	<a href="#">IBITR167-20</a>	
	<i>Allogamus mortoni</i> (Navás, 1907)	INV04793	<a href="#">IBITR297-20</a>	<a href="#">BOLD:AAM3837</a>
		INV04794	<a href="#">IBITR298-20</a>	
		INV04795	<a href="#">IBITR299-20</a>	
	<i>Annitella esparraguera</i> (Schmid, 1952)	INV05963	<a href="#">IBITR422-20</a>	<a href="#">BOLD:AAM4103</a>
	<i>Chaetopteryx atlantica</i> Malicky, 1975	INV05965	<a href="#">IBITR424-20</a>	<a href="#">BOLD:AEC7901</a>
	<i>Drusus berthelemyi</i> Sipahiler, 1992	INV05964	<a href="#">IBITR423-20</a>	<a href="#">BOLD:ACO5446</a>
	<i>Drusus bolivari</i> (McLachlan, 1880)	INV04791	<a href="#">IBITR295-20</a>	<a href="#">BOLD:ACO5618</a>
		INV04792	<a href="#">IBITR296-20</a>	
	<i>Enoicyla pusilla</i> (Burmeister, 1839)	INV04796	<a href="#">IBITR300-20</a>	<a href="#">BOLD:AAO2902</a>
	<i>Grammotaulius submaculatus</i> (Rambur, 1842)	INV02799	<a href="#">IBITR117-20</a>	<a href="#">BOLD:AEC8384</a>
		INV04740	<a href="#">IBITR257-20</a>	
	<i>Halesus radiatus</i> (Curtis, 1834)	INV01836	<a href="#">IBITR083-20</a>	<a href="#">BOLD:AAF7718</a>
		INV02469	<a href="#">IBITR100-20</a>	
		INV04743	<a href="#">IBITR260-20</a>	
		INV03722	<a href="#">IBITR162-20</a>	
	<i>Limnephilus bipunctatus</i> Curtis, 1834	INV02609	<a href="#">IBITR109-20</a>	<a href="#">BOLD:AAA4844</a>
	<i>Limnephilus gadarramicus</i> Schmid, 1955	INV03661	<a href="#">IBITR156-20</a>	<a href="#">BOLD:AEC8200</a>
		INV03664	<a href="#">IBITR159-20</a>	
		INV03946	<a href="#">IBITR170-20</a>	
	<i>Limnephilus hirsutus</i> (Pictet, 1834)	INV03655	<a href="#">IBITR155-20</a>	<a href="#">BOLD:AAE6322</a>
		INV03685	<a href="#">IBITR161-20</a>	
		INV01281	<a href="#">IBITR078-20</a>	
	<i>Limnephilus sparsus</i> Curtis, 1834	INV04258	<a href="#">IBITR171-20</a>	<a href="#">BOLD:AAB6375</a>
		INV01284	<a href="#">IBITR079-20</a>	
		INV03651	<a href="#">IBITR153-20</a>	
		INV03653	<a href="#">IBITR154-20</a>	
		INV04738	<a href="#">IBITR255-20</a>	
	<i>Limnephilus vittatus</i> (Fabricius, 1798)	INV02256	<a href="#">IBITR089-20</a>	<a href="#">BOLD:AAK8602</a>
		INV04739	<a href="#">IBITR256-20</a>	

Family	Taxa	IBI code	BOLD code	BOLD BIN
	<i>Mesophylax aspersus</i> (Rambur, 1842)	INV05478	<a href="#">IBITR338-20</a>	
		INV04573	<a href="#">IBITR199-20</a>	<a href="#">BOLD:AAG5761</a>
		INV04662	<a href="#">IBITR207-20</a>	
		INV04672	<a href="#">IBITR208-20</a>	
	<i>Potamophylax cingulatus</i> (Stephens, 1837)	INV04530	<a href="#">IBITR193-20</a>	
		INV01300	<a href="#">IBITR081-20</a>	<a href="#">BOLD:AAC4985</a>
		INV04746	<a href="#">IBITR263-20</a>	
		INV03662	<a href="#">IBITR157-20</a>	
		INV05388	<a href="#">IBITR329-20</a>	
		INV01299	<a href="#">IBITR080-20</a>	<a href="#">BOLD:ABU7930</a>
		INV02247	<a href="#">IBITR087-20</a>	
	<i>Potamophylax latipennis</i> (Curtis, 1834)	INV02253	<a href="#">IBITR088-20</a>	
		INV02257	<a href="#">IBITR090-20</a>	
		INV02472	<a href="#">IBITR103-20</a>	
		INV02800	<a href="#">IBITR118-20</a>	
	<i>Stenophylax fissus</i> (McLachlan, 1875)	INV04741	<a href="#">IBITR258-20</a>	
		INV03616	<a href="#">IBITR148-20</a>	<a href="#">BOLD:AEC6836</a>
	<i>Stenophylax mucronatus</i> McLachlan, 1880	INV03624	<a href="#">IBITR149-20</a>	
		INV03642	<a href="#">IBITR152-20</a>	
		INV04742	<a href="#">IBITR259-20</a>	
		INV04744	<a href="#">IBITR261-20</a>	<a href="#">BOLD:ABY2452</a>
		INV02900	<a href="#">IBITR119-20</a>	<a href="#">BOLD:AED0879</a>
	<i>Stenophylax permistus</i> McLachlan, 1895	INV02951	<a href="#">IBITR130-20</a>	
	<i>Stenophylax sequax</i> (McLachlan, 1875)	INV02964	<a href="#">IBITR133-20</a>	
		INV04655	<a href="#">IBITR205-20</a>	
		INV04656	<a href="#">IBITR206-20</a>	
		INV04745	<a href="#">IBITR262-20</a>	<a href="#">BOLD:AAI0072</a>
	<i>Stenophylax vibex</i> (Curtis, 1834)	INV02957	<a href="#">IBITR131-20</a>	<a href="#">BOLD:AAE8973</a>
Odontoceridae	<i>Odontocerum albicorne</i> (Scopoli, 1763)	INV00020	<a href="#">IBITR013-20</a>	<a href="#">BOLD:AAB5626</a>
		INV00021	<a href="#">IBITR008-16</a>	



Family	Taxa	IBI code	BOLD code	BOLD BIN
		INV05968	<a href="#">IBITR426-20</a>	
		INV05970	<a href="#">IBITR427-20</a>	
		INV05971	<a href="#">IBITR428-20</a>	
		INV05972	<a href="#">IBITR429-20</a>	
	<i>Odontocerum lusitanicum</i> Malicky, 1975	INV05508	<a href="#">IBITR365-20</a>	<a href="#">BOLD:AEC9755</a>
		INV05501	<a href="#">IBITR359-20</a>	
Philopotamidae	<i>Chimarra marginata</i> (Linnaeus, 1767)	INV02459	<a href="#">IBITR091-20</a>	<a href="#">BOLD:AAO1593</a>
		INV00417	<a href="#">IBITR035-20</a>	
		INV00419	<a href="#">IBITR036-20</a>	
		INV00424	<a href="#">IBITR039-20</a>	
		INV00425	<a href="#">IBITR040-20</a>	
		INV00431	<a href="#">IBITR043-20</a>	
		INV00432	<a href="#">IBITR044-20</a>	
		INV00486	<a href="#">IBITR060-20</a>	
		INV00506	<a href="#">IBITR064-20</a>	
		INV02461	<a href="#">IBITR092-20</a>	
		INV03259	<a href="#">IBITR136-20</a>	
		INV05457	<a href="#">IBITR331-20</a>	
	<i>Philopotamus amphilectus</i> McLachlan, 1884	INV04696	<a href="#">IBITR225-20</a>	<a href="#">BOLD:AED0394</a>
	<i>Philopotamus montanus caurelensis</i> González & Terra, 1979	INV02471	<a href="#">IBITR102-20</a>	<a href="#">BOLD:AAO1570</a>
		INV00334	<a href="#">IBITR032-20</a>	
		INV00336	<a href="#">IBITR033-20</a>	
		INV02465	<a href="#">IBITR096-20</a>	
		INV02476	<a href="#">IBITR107-20</a>	
		INV04694	<a href="#">IBITR223-20</a>	<a href="#">BOLD:AEC7824</a>
		INV04695	<a href="#">IBITR224-20</a>	
	<i>Philopotamus perversus</i> McLachlan, 1884	INV00022	<a href="#">IBITR014-20</a>	<a href="#">BOLD:AAO1569</a>
		INV00023	<a href="#">IBITR015-20</a>	
		INV00024	<a href="#">IBITR016-20</a>	
		INV00025	<a href="#">IBITR017-20</a>	

Family	Taxa	IBI code	BOLD code	BOLD BIN
		INV05487	<a href="#">IBITR347-20</a>	
		INV05835	<a href="#">IBITR403-20</a>	
	<i>Philopotamus variegatus</i> (Scopoli, 1763)	INV00458	<a href="#">IBITR048-20</a>	<a href="#">BOLD:AEC7364</a>
		INV00465	<a href="#">IBITR051-20</a>	
	<i>Wormaldia beaumonti</i> Schmid, 1952	INV01598	<a href="#">IBITR082-20</a>	<a href="#">BOLD:AAO2217</a>
		INV03725	<a href="#">IBITR165-20</a>	<a href="#">BOLD:AAO2216</a>
	<i>Wormaldia corvina</i> (McLachlan, 1884)	INV04698	<a href="#">IBITR226-20</a>	<a href="#">BOLD:ABU5927</a>
		INV04699	<a href="#">IBITR227-20</a>	
		INV05840	<a href="#">IBITR408-20</a>	
		INV05841	<a href="#">IBITR409-20</a>	
	<i>Wormaldia lusitanica</i> González & Botosaneanu, 1983	INV05836	<a href="#">IBITR404-20</a>	<a href="#">BOLD:AAO2217</a>
	<i>Wormaldia occipitalis</i> (Pictet, 1834)	INV05838	<a href="#">IBITR406-20</a>	<a href="#">BOLD:AED0699</a>
		INV05837	<a href="#">IBITR405-20</a>	
		INV05839	<a href="#">IBITR407-20</a>	
	<i>Wormaldia triangulifera</i> McLachlan, 1878	INV04765	<a href="#">IBITR276-20</a>	<a href="#">BOLD:AAH9306</a>
	<i>Wormaldia variegata mattheyi</i> Schmid, 1952	INV04703	<a href="#">IBITR229-20</a>	<a href="#">BOLD:AED0151</a>
		INV04702	<a href="#">IBITR228-20</a>	
		INV03723	<a href="#">IBITR163-20</a>	
		INV03726	<a href="#">IBITR166-20</a>	
Phryganeidae	<i>Agrypnia varia</i> (Fabricius, 1793)	INV03663	<a href="#">IBITR158-20</a>	<a href="#">BOLD:AAE4334</a>
		INV05340	<a href="#">IBITR326-20</a>	
Polycentropodidae	<i>Cyrnus cintranus</i> McLachlan, 1884	INV05500	<a href="#">IBITR358-20</a>	
	<i>Plectrocnemia geniculata</i> McLachlan, 1871	INV05953	<a href="#">IBITR415-20</a>	
	<i>Plectrocnemia laetabilis</i> McLachlan, 1880	INV04295	<a href="#">IBITR184-20</a>	<a href="#">BOLD:AAL4393</a>
		INV02464	<a href="#">IBITR095-20</a>	
		INV04704	<a href="#">IBITR230-20</a>	
		INV04705	<a href="#">IBITR231-20</a>	
		INV05389	<a href="#">IBITR330-20</a>	
	<i>Polycentropus corniger</i> McLachlan, 1884	INV04773	<a href="#">IBITR278-20</a>	<a href="#">BOLD:AAL0051</a>
		INV04772	<a href="#">IBITR277-20</a>	



Family	Taxa	IBI code	BOLD code	BOLD BIN
		INV05475	<a href="#">IBITR335-20</a>	
		INV05483	<a href="#">IBITR343-20</a>	
	<i>Polycentropus flavomaculatus</i> (Pictet, 1834)	INV00503	<a href="#">IBITR062-20</a>	<a href="#">BOLD:AAC0971</a>
		INV00504	<a href="#">IBITR063-20</a>	<a href="#">BOLD:ACR2507</a>
	<i>Polycentropus intricatus</i> Morton, 1910	INV04706	<a href="#">IBITR232-20</a>	<a href="#">BOLD:AAL0054</a>
		INV04707	<a href="#">IBITR233-20</a>	
		INV05489	<a href="#">IBITR349-20</a>	
	<i>Polycentropus kingi</i> McLachlan, 1881	INV04709	<a href="#">IBITR235-20</a>	<a href="#">BOLD:AAL0060</a>
		INV05498	<a href="#">IBITR357-20</a>	
		INV00478	<a href="#">IBITR057-20</a>	
		INV04708	<a href="#">IBITR234-20</a>	
	<i>Polycentropus telifer</i> McLachlan, 1884	INV04417	<a href="#">IBITR185-20</a>	<a href="#">BOLD:AAM0001</a>
Psychomyiidae	<i>Lype auripilis</i> McLachlan, 1884	INV04713	<a href="#">IBITR238-20</a>	<a href="#">BOLD:AAO2229</a>
	<i>Lype phaeopa</i> (Stephens, 1836)	INV00485	<a href="#">IBITR059-20</a>	<a href="#">BOLD:AAC4581</a>
	<i>Paduniella vandeli</i> Decamps, 1965	INV04774	<a href="#">IBITR279-20</a>	<a href="#">BOLD:AAK7667</a>
	<i>Psychomyia fragilis</i> (Pictet, 1834)	INV04775	<a href="#">IBITR280-20</a>	
		INV02695	<a href="#">IBITR112-20</a>	<a href="#">BOLD:AEC7914</a>
	<i>Psychomyia pusilla</i> (Fabricius, 1781)	INV04710	<a href="#">IBITR236-20</a>	<a href="#">BOLD:AAO1607</a>
		INV00427	<a href="#">IBITR042-20</a>	<a href="#">BOLD:AEC8086</a>
		INV00806	<a href="#">IBITR070-20</a>	
		INV02749	<a href="#">IBITR115-20</a>	<a href="#">BOLD:AAO1607</a>
		INV04711	<a href="#">IBITR237-20</a>	
		INV05490	<a href="#">IBITR350-20</a>	
		INV05495	<a href="#">IBITR355-20</a>	<a href="#">BOLD:AEC8086</a>
	<i>Tinodes assimilis</i> McLachlan, 1865	INV00521	<a href="#">IBITR067-20</a>	<a href="#">BOLD:AAF7459</a>
		INV01260	<a href="#">IBITR076-20</a>	
		INV02921	<a href="#">IBITR122-20</a>	
		INV04716	<a href="#">IBITR241-20</a>	
		INV04717	<a href="#">IBITR242-20</a>	
	<i>Tinodes foedellus</i> McLachlan, 1884	INV04714	<a href="#">IBITR239-20</a>	<a href="#">BOLD:AAL9978</a>

Family	Taxa	IBI code	BOLD code	BOLD BIN
		INV04715	<a href="#">IBITR240-20</a>	
	<i>Tinodes maculicornis</i> (Pictet, 1834)	INV05954	<a href="#">IBITR416-20</a>	<a href="#">BOLD:AAF7446</a>
	<i>Tinodes waeneri</i> (Linnaeus, 1758)	INV04777	<a href="#">IBITR282-20</a>	<a href="#">BOLD:AAB9068</a>
		INV00491	<a href="#">IBITR061-20</a>	
		INV01280	<a href="#">IBITR077-20</a>	
		INV04576	<a href="#">IBITR200-20</a>	
		INV04581	<a href="#">IBITR203-20</a>	
		INV04423	<a href="#">IBITR187-20</a>	
		INV04500	<a href="#">IBITR188-20</a>	
		INV04776	<a href="#">IBITR281-20</a>	
Ptilocolepidae	<i>Ptilocolepus extensus</i> McLachlan, 1884	INV04266	<a href="#">IBITR172-20</a>	
		INV04690	<a href="#">IBITR221-20</a>	<a href="#">BOLD:AAL2306</a>
		INV04691	<a href="#">IBITR222-20</a>	
	<i>Ptilocolepus granulatus</i> (Pictet, 1834)	INV05967	<a href="#">IBITR425-20</a>	
Rhyacophilidae	<i>Rhyacophila adjuncta</i> McLachlan, 1884	INV00035	<a href="#">IBITR018-20</a>	<a href="#">BOLD:AAD5575</a>
		INV00330	<a href="#">IBITR031-20</a>	
		INV04677	<a href="#">IBITR209-20</a>	
		INV04678	<a href="#">IBITR210-20</a>	
	<i>Rhyacophila dorsalis</i> (Curtis, 1834)	INV04811	<a href="#">IBITR314-20</a>	<a href="#">BOLD:AAC4103</a>
		INV05793	<a href="#">IBITR371-20</a>	
		INV05794	<a href="#">IBITR372-20</a>	
		INV05795	<a href="#">IBITR373-20</a>	
	<i>Rhyacophila dorsalis albarracina</i> Malicky, 2002	INV05790	<a href="#">IBITR368-20</a>	
		INV05791	<a href="#">IBITR369-20</a>	
	<i>Rhyacophila evoluta</i> McLachlan, 1879	INV04807	<a href="#">IBITR310-20</a>	<a href="#">BOLD:AAX8713</a>
	<i>Rhyacophila intermedia</i> McLachlan, 1868	INV04680	<a href="#">IBITR211-20</a>	
		INV05507	<a href="#">IBITR364-20</a>	
		INV04810	<a href="#">IBITR313-20</a>	<a href="#">BOLD:AAF7929</a>
	<i>Rhyacophila laevis</i> Pictet, 1834	INV04809	<a href="#">IBITR312-20</a>	<a href="#">BOLD:AAF8011</a>
	<i>Rhyacophila laufferi</i> Navás, 1918	INV05800	<a href="#">IBITR378-20</a>	



Family	Taxa	IBI code	BOLD code	BOLD BIN
	<i>Rhyacophila lusitanica</i> McLachlan, 1884	INV02967	<a href="#">IBITR134-20</a>	<a href="#">BOLD:AEC8059</a>
		INV00039	<a href="#">IBITR019-20</a>	
		INV03379	<a href="#">IBITR138-20</a>	
		INV03633	<a href="#">IBITR150-20</a>	
		INV05504	<a href="#">IBITR362-20</a>	
	<i>Rhyacophila martynovi</i> Mosely, 1930	INV05799	<a href="#">IBITR377-20</a>	
		INV04808	<a href="#">IBITR311-20</a>	<a href="#">BOLD:AEC7148</a>
	<i>Rhyacophila melpomene</i> Malicky, 1976	INV04681	<a href="#">IBITR212-20</a>	<a href="#">BOLD:AEM0544</a>
		INV04682	<a href="#">IBITR213-20</a>	
	<i>Rhyacophila meridionalis</i> Pictet, 1865	INV04683	<a href="#">IBITR214-20</a>	<a href="#">BOLD:AEC9268</a>
		INV04684	<a href="#">IBITR215-20</a>	
	<i>Rhyacophila mocsaryi tredosensis</i> Schmid, 1952	INV05796	<a href="#">IBITR374-20</a>	<a href="#">BOLD:AEC7310</a>
	<i>Rhyacophila munda</i> McLachlan, 1862	INV05803	<a href="#">IBITR379-20</a>	<a href="#">BOLD:AAM4449</a>
		INV05804	<a href="#">IBITR380-20</a>	
		INV05805	<a href="#">IBITR381-20</a>	
		INV02949	<a href="#">IBITR129-20</a>	<a href="#">BOLD:AEC7678</a>
		INV03535	<a href="#">IBITR139-20</a>	
		INV04572	<a href="#">IBITR198-20</a>	<a href="#">BOLD:AAM4448</a>
		INV04577	<a href="#">IBITR201-20</a>	<a href="#">BOLD:AEC7678</a>
		INV03900	<a href="#">IBITR168-20</a>	
		INV04420	<a href="#">IBITR186-20</a>	
	<i>Rhyacophila nevada</i> Schmid, 1952	INV05792	<a href="#">IBITR370-20</a>	
	<i>Rhyacophila obelix</i> Malicky, 1979	INV00044	<a href="#">IBITR020-20</a>	<a href="#">BOLD:AEC8711</a>
		INV00045	<a href="#">IBITR021-20</a>	<a href="#">BOLD:AEC8521</a>
		INV05947	<a href="#">IBITR410-20</a>	<a href="#">BOLD:AEC8711</a>
		INV05949	<a href="#">IBITR411-20</a>	
	<i>Rhyacophila oblitterata</i> McLachlan, 1863	INV05797	<a href="#">IBITR375-20</a>	
		INV05798	<a href="#">IBITR376-20</a>	
	<i>Rhyacophila occidentalis</i> McLachlan, 1879	INV04685	<a href="#">IBITR216-20</a>	<a href="#">BOLD:AAJ3548</a>
		INV04686	<a href="#">IBITR217-20</a>	

Family	Taxa	IBI code	BOLD code	BOLD BIN
	<i>Rhyacophila pascoei</i> McLachlan, 1879	INV04755	<a href="#">IBITR269-20</a>	<a href="#">BOLD:AEC7530</a>
	<i>Rhyacophila pulchra</i> Schmid, 1952	INV04687	<a href="#">IBITR218-20</a>	<a href="#">BOLD:AEC8544</a>
	<i>Rhyacophila relictata</i> McLachlan, 1879	INV05806	<a href="#">IBITR382-20</a>	<a href="#">BOLD:AAI0887</a>
		INV04532	<a href="#">IBITR195-20</a>	
		INV04531	<a href="#">IBITR194-20</a>	
		INV05807	<a href="#">IBITR383-20</a>	
		INV06215	<a href="#">IBITR434-20</a>	
	<i>Rhyacophila sociata</i> Navás 1916	INV05808	<a href="#">IBITR384-20</a>	<a href="#">BOLD:AAD5575</a>
		INV05809	<a href="#">IBITR385-20</a>	
	<i>Rhyacophila terpsichore</i> Malicky, 1976	INV04756	<a href="#">IBITR270-20</a>	<a href="#">BOLD:AEC8427</a>
	<i>Rhyacophila terrai</i> González & Martínez, 2010	INV04757	<a href="#">IBITR271-20</a>	<a href="#">BOLD:AEM3903</a>
		INV04758	<a href="#">IBITR272-20</a>	
	<i>Rhyacophila tristis</i> Pictet, 1834	INV05810	<a href="#">IBITR386-20</a>	<a href="#">BOLD:ABA2486</a>
		INV05811	<a href="#">IBITR387-20</a>	
Sericostomatidae	<i>Schizopelex festiva</i> (Rambur, 1842)	INV00053	<a href="#">IBITR022-20</a>	<a href="#">BOLD:AAI0810</a>
		INV00054	<a href="#">IBITR023-20</a>	
		INV00513	<a href="#">IBITR065-20</a>	
	<i>Sericostoma pyrenaicum</i> Pictet, 1865	INV02747	<a href="#">IBITR113-20</a>	<a href="#">BOLD:AAJ7690</a>
		INV02748	<a href="#">IBITR114-20</a>	
		INV04292	<a href="#">IBITR182-20</a>	
		INV04749	<a href="#">IBITR265-20</a>	
		INV04803	<a href="#">IBITR306-20</a>	
		INV05509	<a href="#">IBITR366-20</a>	<a href="#">BOLD:AEC8551</a>
		INV04288	<a href="#">IBITR178-20</a>	
		INV04289	<a href="#">IBITR179-20</a>	<a href="#">BOLD:AAJ7690</a>
		INV04290	<a href="#">IBITR180-20</a>	<a href="#">BOLD:AEC8551</a>
		INV04291	<a href="#">IBITR181-20</a>	
		INV04293	<a href="#">IBITR183-20</a>	<a href="#">BOLD:ABZ0751</a>
		INV04804	<a href="#">IBITR307-20</a>	<a href="#">BOLD:AAJ7690</a>
		INV04805	<a href="#">IBITR308-20</a>	



Family	Taxa	IBI code	BOLD code	BOLD BIN
		INV04806	<a href="#">IBITR309-20</a>	
		INV05472	<a href="#">IBITR332-20</a>	<a href="#">BOLD:AEC8551</a>
	<i>Sericostoma vittatum</i> Rambur, 1842	INV04270	<a href="#">IBITR176-20</a>	<a href="#">BOLD:ABZ0751</a>
		INV04797	<a href="#">IBITR301-20</a>	<a href="#">BOLD:AAM4952</a>
		INV04822	<a href="#">IBITR320-20</a>	<a href="#">BOLD:AAJ7690</a>
		INV05486	<a href="#">IBITR346-20</a>	<a href="#">BOLD:ABZ0751</a>
		INV05492	<a href="#">IBITR352-20</a>	<a href="#">BOLD:AAJ7690</a>
		INV04752	<a href="#">IBITR266-20</a>	
		INV04798	<a href="#">IBITR302-20</a>	<a href="#">BOLD:AAM4952</a>
		INV04799	<a href="#">IBITR303-20</a>	
		INV04800	<a href="#">IBITR304-20</a>	<a href="#">BOLD:AEC9666</a>
		INV04801	<a href="#">IBITR305-20</a>	<a href="#">BOLD:AAJ7690</a>
		INV04820	<a href="#">IBITR318-20</a>	
		INV04821	<a href="#">IBITR319-20</a>	
Thremmatidae	<i>Thremma gallicum</i> McLachlan, 1880	INV05950	<a href="#">IBITR412-20</a>	<a href="#">BOLD:AAF7946</a>
		INV00056	<a href="#">IBITR009-16</a>	
		INV00057	<a href="#">IBITR010-16</a>	
		INV00058	<a href="#">IBITR011-16</a>	
	<i>Thremma tellae</i> González, 1978	INV04833	<a href="#">IBITR324-20</a>	<a href="#">BOLD:AAL9956</a>
		INV05497	<a href="#">IBITR356-20</a>	

Average nucleotide composition of the Trichoptera sequences is 37.7% thymine (T), 17.9% cytosine (C), 30.5% adenine (A) and 13.9% guanine (G), for a total GC content of 31.8% for the COI barcode fragment analysed. Genetic p-distances ranged from 0.00% between *Athripsodes alentexanus* Martín, González and Martínez, 2016 (n = 2) and *A. braueri* (Pictet, 1865) to 33.97% between *Ptilocolepus granulatus* (Pictet, 1834) (n = 1) and *Potamophylax latipennis* (Curtis, 1834) (n = 4) (Suppl. material 3). Intraspecific genetic p-distances ranged from 0.00% in 12 species, including several species of *Athripsodes*, *Hydropsyche* and *Rhyacophila* (average n = 3.16 specimens per species), to 6.16% in *Hydropsyche pictetorum* Botosaneanu and Schmid, 1973 (n = 4), 6.22% in *Psychomyia pusilla* (Fabricius, 1781) (n = 7), 6.65% in *Rhyacophila munda* McLachlan, 1862 (n = 9) and 7.45% in *Helicopsyche lusitanica* McLachlan, 1884 (n = 2). Forty-seven species were represented by a single specimen in the dataset and, for this reason, no intraspecific distance is calculated.

The BOLD BIN system uses algorithms to cluster sequences into operational taxonomic units (OTUs) that closely correspond to species (Ratnasingham and Hebert 2013). A total of 146 BINs were retrieved by BOLD (Ratnasingham and Hebert 2007). Seven specimens have not been BIN attributed as their sequence is only 418 bp and no other specimens have been sequenced (Suppl. material 1). Two specimens, identified to the genus level only as *Helicopsyche* sp., clustered together in a separate BIN, “[BOLD:AEC8747](#)”. Of the 146 BINs, 45 BINs are unique to our dataset (Table 1, Suppl. material 1). Using the criteria followed by Ratnasingham and Hebert (2013), there were 83.6% of matches, 3.7% of merges, 6.7% of splits and 6.0% of mixtures when comparing BINs to the morphological identifications (Fig. 1). The BINs generated by BOLD clustered together sequences that closely agree with the morphological identifications of the specimens, with only a few exceptions in nine of the 22 Trichoptera families analysed.

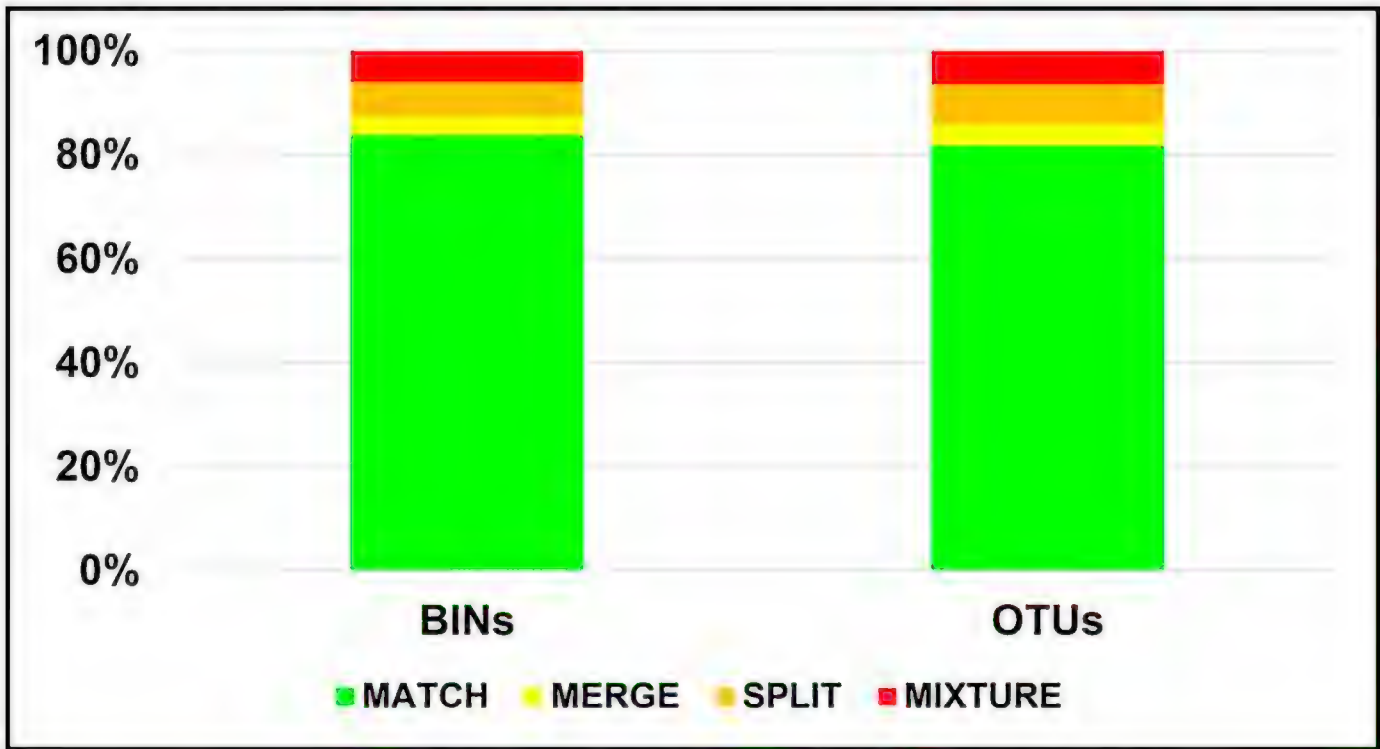


Figure 1. [doi](#)  
Comparison in OTU assignment performance between BOLD’s BIN and RESL stand-alone algorithms. The BIN dataset comprised 135 taxa (134 species) and the RESL stand-alone run comprised the entire 142 taxa (141 species) dataset. The four categories: MATCH, MERGE, SPLIT and MIXTURE into which the OTUs were divided, follow the criteria used by Ratnasingham and Hebert (2013).

The independent RESL run (Ratnasingham and Hebert 2013, Ratnasingham and Hebert 2007) retrieved 153 OTUs, plus one OTU for the *Helicopsyche* sp. specimens (Suppl. material 4). The differences found between the RESL OTUs and the morphological identifications were similar to those found between the latter and BOLD’s BINs, with 81.7% of matches, 4.2% of merges, 7.7% of splits and 6.3% of mixtures when comparing OTUs to the morphological identifications (Fig. 1).

Nevertheless, some differences existed between the RESL OTU clustering and the BINs created by BOLD (Suppl. materials 1, 4). In the family Hydropsychidae, sequences identified as *Hydropsyche instabilis* (Curtis, 1834) clustered into a single OTU, but were



split into two BINs. In the family Leptoceridae, sequences of specimens identified as *Athripsodes alentexanus* and *A. braueri* clustered in a single BIN. In the family Philopotamidae, sequences identified as *Philopotamus perversus* McLachlan, 1884 clustered into two OTUs, but were represented by a single BIN. In the family Polycentropodidae, sequences identified as *Polycentropus flavomaculatus* clustered into a single OTU, but were split into two BINs. In the family Rhyacophilidae, sequences identified as *R. dorsalis* (Curtis, 1834) and its subspecies, *R. d. albarracina* Malicky, 2002 clustered into a single OTU, but other sequences identified as *R. dorsalis* clustered into a different OTU. All *R. dorsalis* sequences share a single BIN, but the subspecies' sequences have not been BIN attributed as their sequences are only 418 bp. Sequences identified as *R. intermedia* McLachlan, 1868 clustered into three OTUs, but were represented by a single BIN. Additionally, sequences identified as *R. martynovi* Mosely, 1930 clustered into two OTUs, but were represented by a single BIN. Furthermore, sequences identified as *Rhyacophila munda* clustered into two OTUs, but were split into three BINs. In the family Sericostomatidae, there was no separation of the species *Sericostoma pyrenaicum* and *S. vittatum*. These species clustered together into two different BINs, but sequences of *S. pyrenaicum* and *S. vittatum* also clustered in additional BINs (Suppl. materials 1, 4).

This work provided new DNA barcode sequences and distributional data for 436 specimens of Iberian Trichoptera, plus two French specimens. The dataset represents 37% of the Caddisflies known to occur in Iberia and the work added 29 taxa previously not represented in the BOLD database. To our knowledge, this is the first study to focus on DNA barcoding of the Trichoptera order for the Iberian Peninsula.

This study showed that DNA barcode sequences, based on the COI mitochondrial gene fragment, can be useful in identifying Iberian Trichoptera samples to species level. We achieved more than 80% success in matching the sequences generated to the morphological identification of the specimens. This is similar to the success rate achieved in 2017 (Morinière et al. 2017) for German Caddisflies (79.8%). A DNA barcode library is an essential tool for incorporating Environmental DNA techniques in monitoring schemes of aquatic ecosystems that use Iberian Caddisflies (Lefrançois et al. 2020). Our results constitute a first step in the construction of a DNA barcode database of a curated reference collection of Iberian Trichoptera species, which could be used to overcome the difficulties in identifying many of the Trichoptera larval specimens of traditional biological freshwater monitoring studies.

Incongruences were found in nine families. In six of them, Glossosomatidae, Helicopsychidae, Polycentropodidae, Limnephilidae, Rhyacophilidae and Psychomyiidae, the barcode analysis identified no species boundaries, with high levels of intraspecific genetic diversity (Suppl. material 3). It is possible that such levels of genetic diversity point to undescribed, distinct species. This hypothesis requires further morphological studies to search for diagnostic morphological traits that might separate these species.

In the family Hydropsychidae, nine species of the genus *Hydropsyche* could be identified through their barcodes and their genetic distances ranged between 13.4% and 23%.

However, five other species could not be identified through DNA barcodes. These species, *H. ambigua*, *H. infernalis*, *H. pictetorum*, *H. siltalai* and *H. tenuis* were spliced between different BINs and OTUs, shared by some, but not all of the same species, further complicating their relationships. For the species with enough sequenced specimens, all were found to have moderate to high levels of intraspecific genetic diversity (Suppl. material 3). These species are difficult to identify morphologically and this study emphasises the need for further work towards a better understanding of the taxonomy of the genus in the Iberian Peninsula (Zamora-Muñoz et al. 2017).

In the family Leptoceridae, sequences identified as *Athripsodes alentexanus* and *A. braueri* clustered in a single BIN. All four sequences were identical. As such, DNA barcodes, based on COI, might not differentiate between these two species. This can be the result of an introgression event, if they had split very recently or alternatively, if their taxonomic identity needs revision.

In the family Philopotamidae, two *Wormaldia beaumonti* and one *W. lusitanica* sequences were in the dataset. Two BINs are present in BOLD with both species represented in each (from previous data, but also with the new data). This genus is very difficult to identify morphologically and is likely that the morphological characters used are not able to separate both taxa.

In the family Sericostomatidae, there were problems separating two species of the genus *Sericostoma*, *S. pyrenaicum* and *S. vittatum*. These species clustered together into two different BINs, but sequences of *S. pyrenaicum* and *S. vittatum* also clustered in additional BINs. Intraspecific genetic diversity is relatively high in both species (2.49% and 2.89%, respectively). González et al. (1992) and Martínez (2014) already pointed out that, under these two names, a complex of species is actually hidden, some of them quite variable morphologically. A detailed morphological-molecular study may help to solve one of the most difficult taxonomic problems of our fauna. These findings suggest that both species need a taxonomic revision.

Our results did not corroborate the findings of Valladolid et al. (2018) and suggest further work is necessary regarding the identity of *Rhyacophila adjuncta* and *R. sociata*. These authors restored the species *R. sociata*, previously considered a junior synonym of *R. denticulata* McLachlan, 1879. However, both BOLD clustering algorithms merged our samples, identified as *R. adjuncta* (2 specimens) and *R. sociata* (2 specimens), into a single BIN "[BOLD:AAD5575](#)". Furthermore, this BIN includes all publicly available sequences in BOLD identified as *R. adjuncta* and *R. sociata*, including all sequences generated by Valladolid et al. (2018). In their paper, the authors did not investigate a possible relationship between these two species, nor was that relationship assessed in a subsequent study on the European species of the *R. fasciata* group (Valladolid et al. 2021). Finally, the BIN mentioned above also includes other sequences identified as *R. tristis* Pictet, 1834. and *R. fasciata* Hagen, 1859, although these are probably misidentifications.

We also identified several cases that require further study by taxonomists. Other possibilities for the incongruence found amongst the results include the existence of



hybridisation, introgression or incomplete lineage sorting in these species, especially if they result from recent speciation events (e.g. Behrens-Chapuis et al. (2021), Morinière et al. (2017), Zhou et al. (2016)). These hypotheses require the combination of nuclear and mitochondrial markers to be resolved, preferably in an integrative taxonomic approach.

## Project description

**Title:** The InBIO Barcoding Initiative Database: DNA Barcodes of Iberian Trichoptera 01

**Personnel:** Luis Martín (taxonomist), Jesús Martínez (taxonomist), Marcos A. González (taxonomist), affiliated to Universidad de Santiago de Compostela; Pedro Beja (project coordinator), Joana Paupério (IBI manager), Sónia Ferreira (taxonomist and IBI manager), Filipa M.S. Martins (molecular biologist), Joana Veríssimo (molecular biologist), Pamela Puppo (molecular biologist), Joana C. Pinto (project technician), Cátia Chaves (project technician), Catarina J. Pinho (project technician), Pedro Sousa (project technician), Lorenzo Quaglietta (ecologist), Teresa Silva (molecular biologist), Paulo Célio Alves, (molecular biologist), Nuno Fonseca (bioinformatician), all affiliated to CIBIO-InBIO, University of Porto and José Manuel Grosso-Silva (taxonomist), affiliated to the MHNC-UP, University of Porto.

**Study area description:** Iberian Peninsula (Fig. 2).

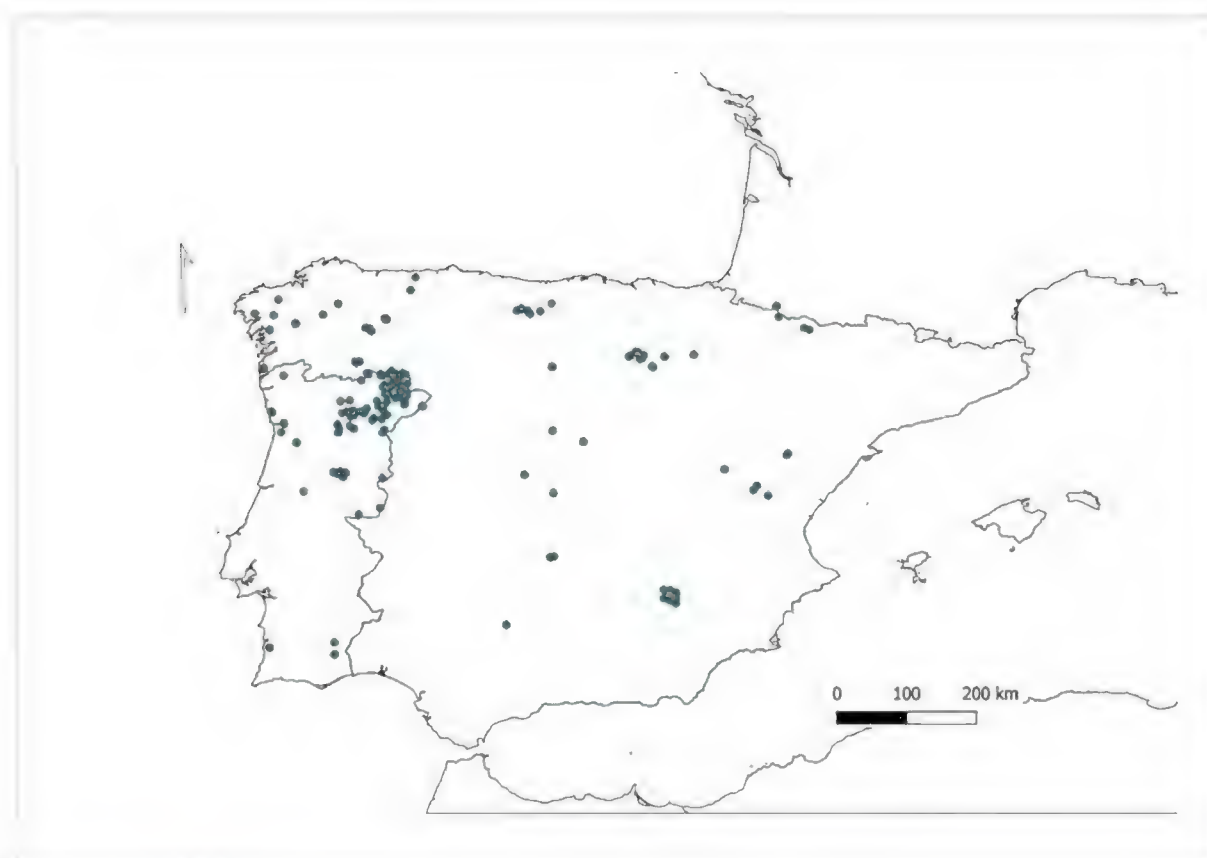


Figure 2. [doi](#)

Sampling localities of the Trichoptera specimens analysed in this study. Nine localities could not be mapped because geographic coordinates were not available.

**Design description:** Specimens were collected during field expeditions in the Iberian Peninsula, from 1975 to 2018 (n = 434 Fig. 2, Suppl. material 1), with more than 60% of

specimens collected in the period between 2015 and 2017 (274 out of 434). Two additional specimens were collected in the French Pyrenees. Specimens kept at the InBIO Barcoding Initiative (IBI) reference collection (Vairão, Portugal), 230 in total, were stored in 96% ethanol. Specimens kept at the Colección Marcos A. González (Universidad de Santiago de Compostela, Spain), 206 in total, were stored in either 70% or 96% ethanol.

For each species, we selected, on average, three specimens for DNA sequencing, based on their location of capture, attempting to maximise the geographical coverage of the study.

DNA was extracted using two different kits: EasySpin Genomic DNA Microplate Tissue Kit (Citomed, Odivelas, Portugal) or QIAmp DNA Micro Kit (Qiagen, Hilden, Germany). QIAmp DNA Micro Kit is designed to extract higher concentrations of genetic material from samples with small amounts of DNA.

DNA amplification was performed using three different primer pairs, that amplify three overlapping fragments of the same 658 bp region of the COI mitochondrial gene. In the beginning of the project (2015), we used two primer pairs, LCO1490 (Folmer et al. 1994) + III\_C\_R (Shokralla et al. 2015) and III\_B\_F (Shokralla et al. 2015) + HCO2198 (Folmer et al. 1994) (henceforth referred to as LC and BH, respectively) to amplify two overlapping fragments of 325 bp and 418 bp, respectively. After the publication of the third primer pair, BF2 + BR2 (422 bp fragment), by (Elbrecht and Leese 2017), this started to be used instead of the second primer pair (III\_B\_F + HCO2198) due to higher amplification efficiency. PCRs were performed in 10 µl reactions, containing 5 µl of Multiplex PCR Master Mix (Qiagen, Germany), 0.3 (BF2-BR2) – 0.4 mM of each primer, and 1-2 µl of DNA, with the remaining volume in water. The thermocycling for PCR reactions was performed in T100 Thermal Cycler (Bio-Rad, California, USA) and carried out with an initial denaturation at 95°C for 15 min, followed by 5 cycles at 95°C for 30 sec, 47°C for 45 sec, 72°C for 45 sec (only for LC and BH); then 40 cycles at 95°C for 30 sec, 51°C for 45 sec (48°C for 60 sec for BF2 + BR2), 72°C for 45 sec; and a final elongation step at 60°C for 10 min.

All PCR products were analysed by agarose gel electrophoresis and samples selected for sequencing were then organised for assignment of sequencing ‘indexes’. One of two types of index was used for each run. For Illumina indexes, samples were pooled into one plate, as described in Shokralla et al. (2015). When using custom indexes, designed, based on Meyer and Kircher (2010), no pooling was required. The latter allow for a maximum of 1920 unique index combinations. A second PCR was then performed where the ‘indexes’ and Illumina sequencing adapters were attached to the PCR product. The index PCR was performed in a volume of 10 µl, including 5 µl of Phusion® High-Fidelity PCR Kit (New England Biolabs, U.S.A.) or KAPA HiFi PCR Kit (KAPA Biosystems, U.S.A.), 0.5 µl of each ‘index’ and 2 µl of diluted PCR product (usually 1:4). This PCR reaction runs for 10 cycles at an annealing temperature of 55°C. The amplicons were purified using AMPure XP beads (Beckman Coulter Genomics, Massachusetts, United States) before quantification using NanoDrop 1000 (Thermo Fisher Scientific, Massachusetts, USA). Concentrations between samples were then normalised and samples were pooled, based on used primer sets.



Quantification of final pools was assessed through qPCR using the KAPA Library Quantification Kit Illumina® Platforms (Kapa Biosystems) and the 2200 TapeStation System (Agilent Technologies, California, USA) was used for fragment length analysis as described by Paupério et al. (2018).

Sequencing was conducted in an Illumina MiSeq benchtop system, using a V2 MiSeq sequencing kit (2x 250 bp) to perform sequencing at CIBIO facilities.

Sequences were filtered and processed with OBITools (Boyer et al. 2015) and the fragments were assembled into their consensus 658 bp-long sequences using Geneious 6.1.8 (<https://www.geneious.com>). The obtained DNA sequences were then compared against the Barcode of Life Data Systems (BOLD) database (Ratnasingham and Hebert 2007) using the built-in identification engine, based on the BLAST algorithm. Sequences were submitted to the BOLD database and the Barcode Index Numbers (BIN) for every sequence were retrieved and analysed (Suppl. materials 1, 2). As not all our sequences matched the criteria used in BOLD (sequence length) to be clustered in a BIN, we ran the Refined Single Linkage algorithm (RESL, Ratnasingham and Hebert (2013)) on our dataset in the BOLD system (Ratnasingham and Hebert 2007) in an independent run (Suppl. material 4). This process clusters sequences independent of their BIN registry, generating OTUs that can be analysed independently.

All DNA barcode sequences were aligned in Geneious 6.1.8 with MUSCLE (Edgar 2004) plug-in. Nucleotide composition of all sequences, as well as intra and interspecific p-distances, were calculated in MEGA11 (Tamura et al. 2021).

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## Sampling methods

**Description:** Iberian Peninsula.

**Sampling description:** Specimens were captured during direct searches of the environment, using mainly hand-held sweep-nets or lured by light trapping, the latter with UV (black-light) LEDs. Morphological identification was done, based on Malicky (2004)

using a stereoscopic microscope for the study of genitalia. In some cases, genitalia were cleared in 10% potassium hydroxide (KOH) at room temperature for 4–8 hours, rinsed in water and placed in a drop of glycerine or resin (DMHF) on a clean slide for further study. From each specimen, one tissue sample (a leg) was removed and stored in 96% ethanol for DNA extraction at the IBI collection.

**Quality control:** All DNA barcode sequences were compared against the BOLD database and the 99 top results were inspected in order to detect possible problems due to contaminations or misidentifications. Prior to GBIF submission, data were checked for errors and inconsistencies with OpenRefine 3.3 (<http://openrefine.org>).

**Step description:** Specimens were collected in 66 different localities in Portugal and 74 localities in Spain. Collections were carried out between 1975 and 2018. Specimens were collected during fieldwork by direct search of specimens, by sweeping the vegetation with a hand-net and by using light traps and were preserved in 96% alcohol. Captured specimens were deposited in the IBI reference collection at CIBIO (Research Center in Biodiversity and Genetic Resources) or in the collection Marcos A. González at the University of Santiago de Compostela (Spain). Specimens were morphologically identified with the assistance of stereoscopic microscopes (Leica MZ12, 8x to 100x; Olympus SZX16, 7x to 115x). DNA barcodes were sequenced from all specimens. For this, one leg was removed from each individual, DNA was then extracted and a 658 bp COI DNA barcode fragment was amplified and sequenced. All obtained sequences were submitted to BOLD and GenBank databases and, to each sequenced specimen, the morphological identification, when available, was contrasted with the results of the BLAST of the newly-generated DNA barcodes in the BOLD Identification Engine. Prior to submission to GBIF, data were checked for errors and inconsistencies with OpenRefine 3.3 (<http://openrefine.org/>).

## Geographic coverage

**Description:** Specimens were collected in the Iberian Peninsula, 229 from 66 localities in Portugal and 207 from 74 localities in Spain (Fig. 2, Suppl. material 5 for further details). Two additional specimens were collected in two French localities. The *Rhyacophila laevis* Pictet, 1834 specimen represented in the dataset was collected in the French Pyrenees.

**Coordinates:** -8.94 and -0.22 Latitude; 42.89 and 37.50 Longitude.

## Taxonomic coverage

**Description:** This dataset is composed of data relating to 438 Trichoptera specimens. All specimens were determined to species level, with 14 specimens further identified to subspecies level. Overall, 141 species are represented in the dataset. These species belong to 22 families.



Taxa included:

Rank	Scientific Name	Common Name
kingdom	Animalia	Animals
subkingdom	Eumetazoa	
phylum	Arthropoda	
class	Insecta	
family	Apataniidae	
family	Beraeidae	
family	Brachycentridae	
family	Calamoceratidae	
family	Ecnomidae	
family	Glossosomatidae	
family	Goeridae	
family	Helicopsychidae	
family	Hydropsychidae	
family	Hydroptilidae	
family	Lepidostomatidae	
family	Leptoceridae	
family	Limnephilidae	
family	Odontoceridae	
family	Philopotamidae	
family	Phryganeidae	
family	Polycentropodidae	
family	Psychomyiidae	
family	Ptilocolepidae	
family	Rhyacophilidae	
family	Sericostomatidae	
family	Uenoidae	

## Temporal coverage

Data range: 1975-5-03 - 2018-5-16.

## Collection data

Collection name: InBIO Barcoding Initiative

Collection identifier: 4ec2b246-f5fa-4b90-9a8d-ddafc2a3f970

Specimen preservation method: "Alcohol"

Curatorial unit: DNA extractions - 1 to 438

## Usage licence

Usage licence: Creative Commons Public Domain Waiver (CC-Zero)

## Data resources

Data package title: [The InBIO Barcoding Initiative Database: DNA Barcodes of Iberian Trichoptera](#)

Resource link: <http://dx.doi.org/10.5883/DS-IBITR01>

Number of data sets: 1

Data set name: DS-IBITR01 IBI-Trichoptera 01

Download URL: [http://www.boldsystems.org/index.php/Public\\_SearchTerms?query=DS-IBITR01](http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-IBITR01)

Data format: dwc, xml, tsv, fasta

**Description:** The InBIO Barcoding Initiative Database: DNA Barcodes of Iberian Trichoptera dataset can be downloaded from the PublicData Portal of BOLD ([http://www.boldsystems.org/index.php/Public\\_SearchTerms?query=DS-IBITR01](http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-IBITR01)) in different formats (data as dwc, xml or tsv and sequences as fasta files). Alternatively, BOLD users can log-in and access the dataset via the Workbench platform of BOLD. All records are also searchable within BOLD, using the research function of the database. The InBIO Barcoding Initiative will continue sequencing Iberian Trichoptera for the BOLD database, with the ultimate goal of comprehensive coverage. The version of the dataset, at the time of writing the manuscript, is included as in the form of one text file for record information as downloaded from BOLD, one text file with the collection and identification data in Darwin Core Standard format (downloaded from GBIF, Martín et al. (2022)) and of a fasta file containing all sequences as downloaded from BOLD. It



should be noted that, as the BOLD database is not compliant with the Darwin Core Standard format, the Darwin Core formatted file (dwc) that can be downloaded from BOLD is not strictly Darwin Core formatted. For a proper Darwin Core formatted file, see [http://ipt.gbif.pt/ipt/resource?r=ibi\\_trichoptera\\_01&v=1.1](http://ipt.gbif.pt/ipt/resource?r=ibi_trichoptera_01&v=1.1) (Suppl. material 5). All data are available in the BioStudies database (<http://www.ebi.ac.uk/biostudies>) under accession number S-BSST920.

Column label	Column description
processid	Unique identifier for the sample.
sampleid	Identifier for the sample being sequenced, i.e. IBI catalogue number at Cibio-InBIO, Porto University. Often identical to the "Field ID" or "Museum ID".
recordID	Identifier for specimen assigned in the field.
catalognum	Catalogue number.
fieldnum	Field number.
institution_storing	The full name of the institution that has physical possession of the voucher specimen.
bin_uri	Barcode Index Number system identifier.
phylum_taxID	Phylum taxonomic numeric code.
phylum_name	Phylum name.
class_taxID	Class taxonomic numeric code.
class_name	Class name.
order_taxID	Order taxonomic numeric code.
order_name	Order name.
family_taxID	Family taxonomic numeric code.
family_name	Family name.
subfamily_taxID	Subfamily taxonomic numeric code.
subfamily_name	Subfamily name.
genus_taxID	Genus taxonomic numeric code.
genus_name	Genus name.
species_taxID	Species taxonomic numeric code.
species_name	Species name.
identification_provided_by	Full name of primary individual who assigned the specimen to a taxonomic group.
identification_method	The method used to identify the specimen.
voucher_status	Status of the specimen in an accessioning process (BOLD controlled vocabulary).
tissue_type	A brief description of the type of tissue or material analysed.

collectors	The full or abbreviated names of the individuals or team responsible for collecting the sample in the field.
lifestage	The age class or life stage of the specimen at the time of sampling.
sex	The sex of the specimen.
lat	The geographical latitude (in decimal degrees) of the geographic centre of a location.
lon	The geographical longitude (in decimal degrees) of the geographic centre of a location.
elev	Elevation of sampling site (in metres above sea level).
country	The full, unabbreviated name of the country where the organism was collected.
province_state	The full, unabbreviated name of the province ("Distrito" in Portugal) where the organism was collected.
region	The full, unabbreviated name of the municipality ("Concelho" in Portugal) where the organism was collected.
exactsite	Additional name/text description regarding the exact location of the collection site relative to a geographic relevant landmark.

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## Supplementary materials

### Suppl. material 1: IBI-Trichoptera 01 library - Specimen details [doi](#)

**Authors:** Joana Paupério, Luis Martín, Sónia Ferreira, Jesús Martínez, Marcus A. González, Martin Corley, José Manuel Grosso-Silva, Lorenzo Quaglietta, Pedro Sousa, Pedro Beja

**Data type:** Record information - specimen data

**Brief description:** The file includes information about all records in BOLD for the IBI-Trichoptera 01 library. It contains collection and identification data. The data are as downloaded from BOLD, without further processing.

[Download file](#) (180.30 kb)

### Suppl. material 2: IBI-Trichoptera 01 library - DNA sequences [doi](#)

**Authors:** Joana Paupério, Luis Martín, Sónia Ferreira, Jesús Martínez, Marcus A. González, Filipa M.S. Martins, Joana Veríssimo, Pamela Puppo, Joana C. Pinto, Cátia Chaves, Catarina Pinho, Pedro Sousa, Pedro Beja

**Data type:** Genomic data, DNA sequences

**Brief description:** COI sequences in fasta format. Each sequence is identified by the BOLD ProcessID, species name, marker and GenBank accession number, separated by pipe. The data are as downloaded from BOLD.

[Download file](#) (298.19 kb)

### Suppl. material 3: Genetic Distances [doi](#)

**Authors:** Joana Paupério, Luis Martín, Sónia Ferreira, Jesús Martínez, Marcus A. González, Filipa M.S. Martins, Joana Veríssimo, Pamela Puppo, Joana C. Pinto, Cátia Chaves, Catarina Pinho, José Manuel Grosso-Silva, Lorenzo Quaglietta, Pedro Sousa, Paulo Célio Alves, Nuno Fonseca, Pedro Beja

**Data type:** Genetic distances between analysed specimens

**Brief description:** Brief description: Estimates of average genetic divergence (uncorrected p-distances) for species of Trichoptera. Values under the diagonal refer to interspecific divergence, while values in the diagonal represent intraspecific divergence.

[Download file](#) (127.58 kb)

### Suppl. material 4: OTUs generated by the Refined Single Linkage algorithm (RESL,) [doi](#)

**Authors:** Joana Paupério, Luis Martín, Sónia Ferreira, Jesús Martínez, Marcus A. González, Filipa M.S. Martins, Joana Veríssimo, Pamela Puppo, Joana C. Pinto, Cátia Chaves, Catarina Pinho, José Manuel Grosso-Silva, Lorenzo Quaglietta, Pedro Sousa, Paulo Célio Alves, Nuno Fonseca, Pedro Beja

**Data type:** OTUs generated by the RESL algorithm and respective sequence composition

**Brief description:** OTUs generated by the RESL algorithm (Ratnasingham and Hebert, 2013) in the BOLD system (Ratnasingham and Hebert, 2007), respective sequence composition and Nearest Neighbour genetic distance. The data are downloaded from BOLD, without further processing.

[Download file](#) (43.36 kb)

**Suppl. material 5: IBI-Trichoptera 01 library - Specimen details - Darwin Core Standard** [doi](#)

**Authors:** Luis Martín, Sónia Ferreira, Jesús Martínez, Marcus A. González, Martin Corley, José Manuel Grosso-Silva, Lorenzo Quaglietta, Pedro Sousa, Pedro Beja

**Data type:** Record information - specimen data in Darwin Core Standard format

**Brief description:** The file includes information about all records in BOLD for the IBI-Trichoptera 01 library. It contains collection and identification data. The data are downloaded from GBIF, without further processing.

[Download file](#) (442.98 kb)